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I. Membrane Potentials and Permeability

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HISTORICAL REVIEW

Resting Membrane Potential

It has been known since Galvani that it is possible to measure electrical potential differences between tissues and across multicellular membranes of plants and animals. More recently, especially since the development of microelectrodes with tips less than 1 μ in diameter, electrical potential differences have been found across cell membranes of individual cells wherever such measurements are possible. This resting membrane potential in many cases corresponds to the electrical potential expected from the asymmetric K^+ ion distribution across the cell membrane according to the Nernst equation:

$$E_m = (2.3RT)/F \log (f_0K^+_o)/(f_iK^+_i) \quad (1)$$

where E_m is the resting membrane potential (in volts); R, the gas constant (1.98 cal/mole degree); T, the absolute temperature; F, the Faraday (23,062 cal/v); K^+_i, K^+_o, f_i, and f_o are the K^+ ion concentrations and their respective activity coefficients inside and outside the cell; and 2.3 is the numerical constant for conversion from $\log_e$ to $\log_{10}$. If it is assumed that the activity coefficient is the same inside and outside the cell (e.g., there is not significant binding of K^+ ions on either side of the membrane), then equation 1 becomes:

$$E_m = (2.3RT)/F \log K^+_o/K^+_i \quad (2)$$

[1] This symposium was held at the Annual Meeting of the American Society for Microbiology, Atlantic City, N.J., 26 April 1965, with R. L. Starkey as convener and consultant editor.

The possibility that the activity coefficients for the K^+ ions on the two sides of the membrane are *not* the same, because of intracellular K^+ binding, and the alternative interpretations of the membrane potential which such binding requires, will be discussed in a later section.

A qualitative statement of the Nernst equation is simply that an electrical potential difference exists across a membrane if there is an asymmetric ionic distribution. Quantitatively, the membrane potential is equal to the product of $(2.3RT)/F$, which is a constant at a given temperature, and the log of the K^+ ion concentration ratio. In the biological range, the constant $(2.3RT)/F$ is approximately equal to 60 mv, e.g., 59.15 mv at 25 C and 61.73 mv at 38 C (11, 28).

In most cells, the intracellular K^+ ion concentration is far greater than the extracellular concentration (19): a K^+_o/K^+_i ratio of 1:10 would represent an $E_m = -60$ mv, whereas a ratio of 1:100 represents an $E_m = -120$ mv. The notion that the membrane potential can be interpreted as a potassium concentration or diffusion potential is supported by the observation that, within limits, in certain muscles and algae (where the concept applies best), the membrane potential changes in conformance with the Nernst equation when the K^+ ion distribution ratio is experimentally altered (2, 4, 19).

The thermodynamic basis of the correspondence between the E_m and the potassium ion distribution expressed by the Nernst equation may be reviewed profitably at this point, since it will place this observation in the proper perspective for the discussion to follow.

The asymmetry of the K^+ ion across the cell membrane represents a chemical potential difference. If K^+ were a nonionized solute, the magnitude of the chemical potential difference would be a measure of the work required to bring about the observed asymmetry. The chemical potential difference $\Delta\mu_{chem}$, associated with the concentration difference across the cell membrane, is (18):

$$\Delta\mu_{chem} = 2.3RT \log K^+_i/K^+_o \quad (3)$$

However, because K^+ is a charged particle, any conclusions about the work required to bring about a given concentration difference across the cell membrane must take into account the electrical potential difference across the membrane (29). The latter represents the work done in transferring a unit charge across the membrane. The electrical potential difference, $\Delta\mu_{elec}$, is given by the product E_mF:

$$\Delta\mu_{elec} = E_mF \quad (4)$$

The electrochemical potential difference, $\Delta\mu$, of a charged particle is, therefore, the sum of its chemical potential difference and its electrical potential difference, as given in equation 5. The electrochemical potential difference for K^+ ion is given in equation 6.

$$\Delta\mu = \Delta\mu_{chem} + \Delta\mu_{elec} \quad (5)$$

$$\Delta\mu = 2.3RT \log K^+_i/K^+_o + E_mF \quad (6)$$

If the K^+ ion distribution across the membrane is the result of a passive process, there will be no electrochemical difference across the membrane at equilibrium (i.e., $\Delta\mu = 0$ for a passive process). In such a case, equation 6 becomes:

$$E_mF = -2.3RT \log K^+_i/K^+_o \quad (7)$$

Equation 7 can be recognized as a form of the Nernst equation (equation 2).

To state that the distribution of an ion conforms to the Nernst equation implies, therefore, that its distribution is the result of a passive process.

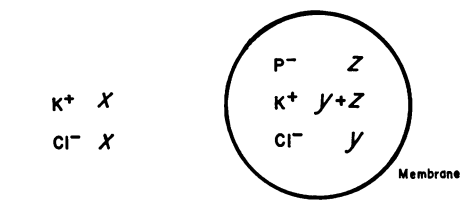
Donnan Equilibrium

The fact that the potassium ion distribution, in those cases in which it conforms with the Nernst equation, is the result of a passive process leaves unexplained the origin of the asymmetry. An early explanation for the K^+ asymmetry was proposed by Bernstein (19), who attributed the K^+ ion asymmetry to a Donnan equilibrium resulting from the presence of intracellular nondiffusible anions (such as proteins). The way that an asymmetry of diffusible ions results from an asymmetry of nondiffusible ions is illustrated in Fig. 1.

A Donnan equilibrium for a nonrigid cell, however, will not actually reach equilibrium because at all times the internal osmotic pressure will be greater than that of the medium. As a result, water tends to move into the cell constantly, which alters the ionic concentrations and causes continuous ion and water movements until the cell bursts (unless it is opposed by a physical or hydrostatic force). A nonpenetrating ion with a charge opposite that of the nondiffusible ion inside the cell would set up an opposing Donnan equilibrium, thus stabilizing such a system. It was long believed that extracellular Na^+ served this function, since the Na^+ is usually present at a higher concentration outside the cell than inside and, unlike the response of the resting membrane potential in certain cells to changes in K^+ ion concentration ratio, the resting membrane potential of these cells is not affected by changes in the Na^+ ion concentration ratio over the same range.

Ion "Pumps"

This view of Na^+ ion impermeability prevailed unmodified until the availability of the radioisotope Na^{24}, when it was found that cells postulated to be impermeable to Na^+ ion were in fact freely permeable to Na^{24}. To account for the apparent impermeability of the cell membrane to Na^+ ions, and for the correspondence between the membrane potential and the K^+ diffusion potential,



$$[K^+]_o[Cl^-]_o = [K^+]_i[Cl^-]_i$$
$$x^2 = y(y+z)$$
$$x^2 = y^2 + yz \quad \therefore x > y = [Cl^-]_o > [Cl^-]_i$$
$$x = \frac{y(y+z)}{x} \quad \therefore (y+z) > x = [K^+]_i > [K^+]_o$$

FIG. 1. *Donnan equilibrium. It is proposed that the membrane surrounding a cell is impermeable to the large anions, P^-, but freely permeable to K^+ and Cl^- ions. Electroneutrality must exist on each side of the membrane at all times. At equilibrium the product $K^+_oCl^-_o = K^+_iCl^-_i$. If the external concentrations of K^+ and Cl^- are represented by X, and the internal concentrations by Y and Z as shown, at equilibrium there will be an asymmetry of K^+ and Cl^- ions across the membrane.*

Dean (19) in 1941 proposed that the Donnan distribution of K^+ ion and, therefore, the K^+ diffusion potential would not be altered if the sodium ion were expelled from the cell in a nonionic form as rapidly as it entered by diffusion from the external medium. Although sodium efflux in a supposedly complexed nonionic form would not affect the membrane potential determined by the potassium ion distribution ratio, sodium efflux would be an energy-dependent process, since Na^+ ion would be distributed against its electrochemical potential gradient (4, 10, 12, 18, 19, 30).

This proposal that metabolic energy is coupled to a process for the maintenance of Na^+ ion against its electrochemical potential gradient is now familiar as the "sodium pump theory." (The quantitative aspects of this energy requirement will be explored for a specific case below.)

The fact that cell membranes are permeable to Na^+ ions, as revealed by the use of Na^{24}, requires that the Nernst equation relating the membrane potential to ionic distribution must take into account not only the K^+ ion but the Na^+ and Cl^- ions as well. The requisite equation without modification would be:

$$E_m = (2.3RT)/F \log \cdot (K^+_i + Na^+_i + Cl^-_o)/(K^+_o + Na^+_o + Cl^-_i) \quad (8)$$

However, Hodgkin and Katz (12) proposed that the equation, to be applied appropriately to a biological system, must take into account for each ion its mobility in the membrane, v, and its partition coefficient, b, between the membrane and the aqueous solutions, which determines the ion concentration in the membrane. The appropriate equation becomes:

$$E_m = (2.3RT)/F \log \cdot (P_K K^+_i + P_{Na} Na^+_i + P_{Cl} Cl^-_o)/(P_K K^+_o + P_{Na} K^+_o + P_{Cl} Cl^-_i) \quad (9)$$

where P_K, P_{Na}, and P_{CL} are permeability coefficients for each ion defined by:

$$P = (RT)/(aF)vb \quad (10)$$

where R, T, and F have their usual meaning, a is the thickness of the membrane, and v and b are the mobility and partition coefficients, respectively, for any given ion. (For derivation of this equation, see reference 12, appendix.) Some of the values for the permeability coefficients (P_K, P_{Na}, and P_{CL}) used in the literature for muscle and nerve are: in frog sartorius, 1, 0.027, and 0.23, respectively; in several muscles of South American frogs, 1, 0.015, and 0.17; and in the giant axon of the squid, 1, 0.04, and 0.45 (12, 19).

When P_{Na} is small relative to P_K, which is true in all of the cases mentioned above, equation 10 reduces to equation 11, which is another form of the Nernst equation describing the diffusion potential which would be observed if only K^+ and Cl^- ions penetrated the membrane.

$$E_m = (2.3RT)/F \log (P_K K^+_i + P_{Cl} Cl^-_o)/(P_K K^+_o + P_{Cl} Cl^-_i) \quad (11)$$

This equation is formally identical with equation 1. However, there are numerous data from several cell types that place some restrictions on the applicability of equation 10 or 11 to membrane potentials. Among the most important observed inconsistencies are the following: the range of external K^+ concentrations over which the E_m of muscle cells obeys the Nernst equation is at concentrations greater than the normal in vivo plasma level; whereas alterations of the external potassium concentration (within the limits just listed) has the predicted effect on the membrane potential, corresponding alterations of the internal concentrations do not give the predicted response; replacement of the axoplasm of the giant axon of the squid with seawater has relatively little effect on the membrane potential. These and other inconsistencies have led to modifications of the membrane theory, reviewed by Ridge and Walker (19).

Finally, although the measured resting mem-

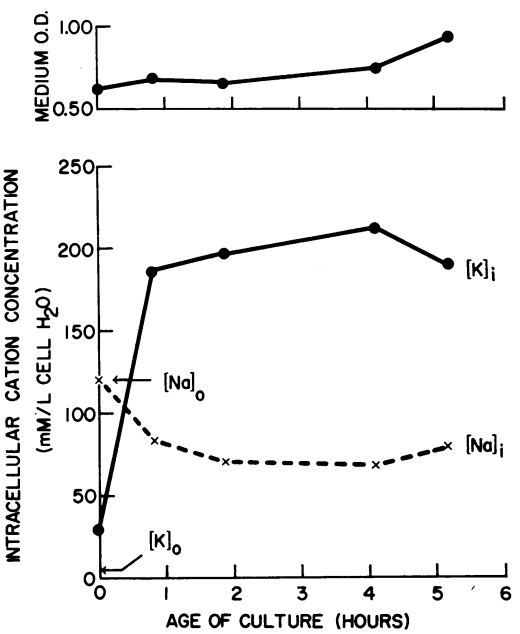


FIG. 2. *Ion distributions between the cell and medium in Escherichia coli during growth. The cellular concentrations of K^+ and Na^+ are shown. The external concentrations of these ions are indicated by the arrows. The optical density changes shown in the upper panel are a measure of cell growth (24).*

TABLE 1. *Intracellular Cl, Na, and K concentrations in Escherichia coli**

Phase of growth	Cl_o	Cl_i	Cl_o/Cl_i	Na_o	Na_i	Na_i/Na_o	K_o	K_i	K_i/K_o
	mM	mM		mM	mM		mM	mM	
Stationary	25	21.2 ± 0.6 (8)†	1.18	145	177 ± 4 (4)	1.22	5	11.2 ± 1.0 (4)	2.2
	50	44.5 ± 2.0 (8)	1.12	170	188 ± 3 (4)	1.11	5	9.6 ± 1.1 (4)	1.9
	75	68.1 ± 3.6 (15)	1.10	145	165 ± 4 (8)	1.14	5	13.0 ± 1.1 (8)	2.6
	100	69.9 ± 1.0 (7)	1.43	170	148 ± 1 (4)	0.85	5	28.3 ± 0.5 (4)	5.7
	Avg		1.13			1.16			2.2
Logarithmic	25	8.3 ± 1.5 (14)	3.0	145	81 ± 2 (7)	0.56	5	217 ± 5 (7)	43.4
	50	17.1 ± 0.9 (27)	2.9	120	70 ± 2 (7)	0.58	5	224 ± 8 (6)	44.8
	75	23.7 ± 3.0 (19)	3.2	145	80 ± 4 (6)	0.55	5	227 ± 5 (7)	45.4
	100	33.5 ± 1.7 (8)	3.0	170	89 ± 8 (4)	0.52	5	257 ± 1 (4)	51.5
	Avg		3.0			0.55			46.3

* The errors shown are the standard errors of the mean; the number of determinations is given in parentheses.

† Cl_i was determined by use of Cl^{36}.

TABLE 2. *Electrochemical potential differences associated with ion distributions in Escherichia coli*

$$\Delta\mu = 2.3RT \log C_i/C_o + FE_m$$
$$\Delta\mu = 2.3RT/F \log C_i/C_o + E_m$$
$$E_m = 2.3RT/F \log Cl^-{}_i/Cl^-{}_o = -26 \text{ mv}$$

Ion	C_i/C_o*	Diffusion potential	E_m	$\Delta\mu/F$
Na^+	0.55	−20	−26	−46
K^+	46.0	+100	−26	+74

* Cation concentration ratio.

brane potentials correspond to K^+ diffusion potentials suggesting that the K^+ ion distribution is the result of a passive process, in other cases both the K^+ and the Na^+ ions are distributed against their electrochemical gradients. In such instances, both K^+ and Na^+ pumps are postulated to account for the electrochemical potential differences represented by their distributions (29, 30).

IONIC DISTRIBUTION IN MICROORGANISMS

Ionic Distribution in Escherichia coli, Interpreted by the Membrane Theory

In a recent series of studies, Schultz et al. (23–25) measured the cell-to-medium distribution of K^+, Na^+, and Cl^- ions in *E. coli* during growth (Fig. 2, Table 1). The ionic distributions observed during the log phase are similar to those observed in higher cells, namely, a high cell-to-medium ratio of K^+ ion and a low cell-to-medium ratio for Na^+ and Cl^- ions. To decide whether any, or all, of these ionic distributions are the result of a passive process or depend upon a "pump," it would be necessary to know the resting membrane potential. In *E. coli* it is not possible to measure this by internal electrodes. However, an indirect approach is possible, based on the concept, reviewed in the previous sections, that the membrane potential represents a diffusion potential of an ion whose asymmetry results from a Donnan equilibrium. This indirect approach involves the calculation of the diffusion potential represented in turn by the K^+, Na^+, and Cl^- ion (Table 2). This calculation gives three different values: −26 mv for Cl^-, −100 mv for K^+, and +20 mv for Na^+. If it is assumed that only one of these diffusion potentials corresponds to the resting membrane potential, and that the related ion is, therefore, distributed by a passive process, then the other two ions are distributed against their electrochemical gradients and must be transported by an "active" process. Schultz et al. presented evidence which indicated that, in the log phase of *E. coli*, the chloride ion is distributed by a passive process. If this is so, then both K^+ and Na^+ ions are transported against electrochemical gradients by an energy-dependent process. The magnitude of the electrochemical potential represented by the K^+ and the Na^+ ion distributions and, hence, a measure of the energy requirements to maintain this potential, may be calculated from the definition of the electrochemical potential which, for K^+ ion, is indicated by equation 6.

Randall (18) suggested that, as a simple computational method to arrive at a measure of the electrochemical potential represented by a given ionic distribution, both sides of the equation be divided by F, to give an equation in the form:

$$(\Delta\mu)/F = (2.3RT)/F \log K^+{}_i/K^+{}_o + E_m \quad (12)$$

For the biological range:

$$(\Delta\mu)/F = 60 \text{ mv} \log K^+{}_i/K^+{}_o + E_m \quad (13)$$

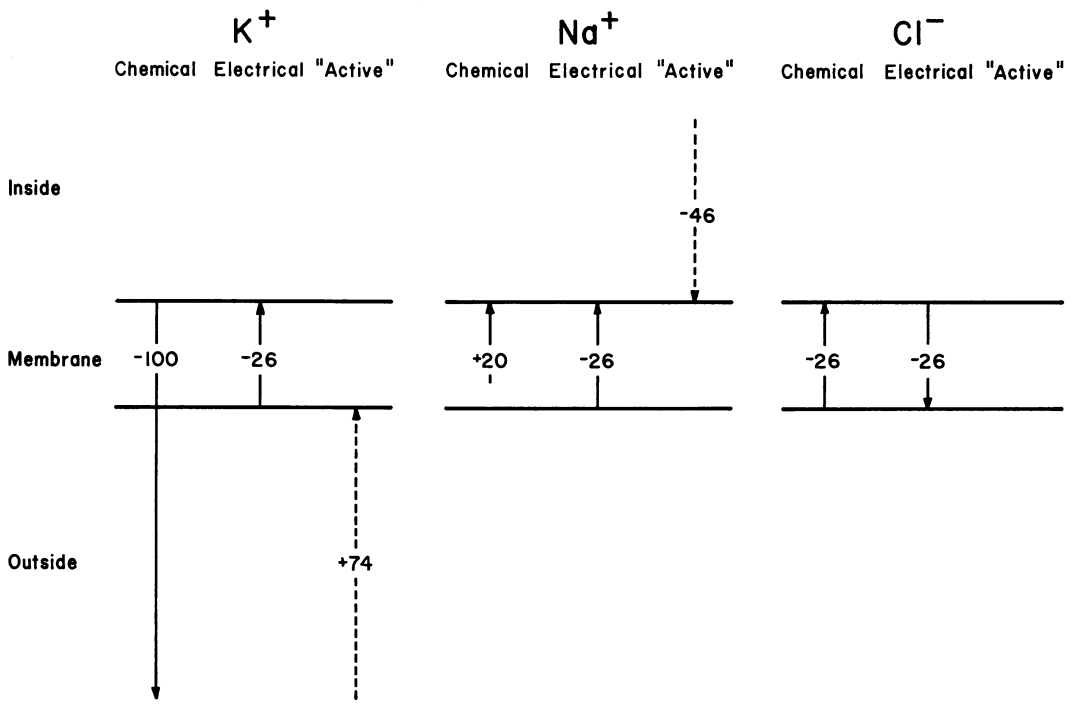


FIG. 3. *Potential differences associated with K^+, Na^+, and Cl^- ion distributions in log-phase cells of Escherichia coli. The data in Table 2 are shown in graphic form. The electrical potential difference, −26 mv, is calculated from the Cl^- ion distribution. The chemical potentials represent the sign and magnitude, in millivolts, expected from the distribution of K^+, Na^+, and Cl^- ions, respectively, if each ion were distributed passively across the membrane in accordance with the Nernst equation. The dotted lines represent the magnitude of the difference between the calculated potential and the chloride potential. Assuming that the chloride potential is the actual membrane potential, the magnitude of the difference and its direction indicate the work required to establish the observed electrochemical potential differences. The work requirement ($\Delta\mu/F$ of equation 12) represents "active" transport. For K^+, the active transport is directed into the cell; for Na^+, it is directed outward. This representation is adapted from Randall (18).*

The term, $(\Delta\ \mu)/F$, is a measure in millivolts of the total electrochemical potential; the middle term is a measure of the chemical potential and is simply the negative of the diffusion potential; the last term is a measure of the electrical potential, and is the membrane potential. This calculation for K^+, Na^+, and Cl^- ions for log-phase *E. coli* is shown in Table 2 and Fig. 3.

These calculations are predicated on two assumptions: that the membrane potential is a diffusion potential, and that the chloride ion is passively distributed across the membrane according to the Nernst equation. The second assumption may be wrong, because the chloride ion may also be distributed against its electrochemical gradient. Without knowledge of the actual membrane potential, this cannot be ascertained. More will be said about the first assumption later.

Support for the idea that both K^+ and Na^+ ions are transported by active processes comes from a study of the characteristics of the distribution of these ions when stationary-phase cells of *E. coli* are inoculated into fresh medium (Fig. 2). The cells taken from the stationary phase at a low *p*H show a cell-to-medium distribution ratio for all these ions of nearly 1 (Table 1). On the basis of the Cl^- ion distribution, the E_m has fallen from −26 mv of the log phase to −6 mv. When such cells are placed in fresh medium at *p*H 7.0, there is an immediate accumulation of intracellular K^+ and an extrusion of the Na^+ ion. Within 1 hr, the log-phase distributions are reached (Fig. 2). The effect of various conditions and inhibitors on the ionic movements is shown in Fig. 4. The metabolic dependence of these net movements, as shown by these data, is expected. The differential inhibition by NaF of the Na and K net fluxes, however, is particularly noteworthy, since in many cells the movements of these two ions is tightly coupled. Inhibition of the flux of one has a marked effect on the flux of the other (10). In *E.*

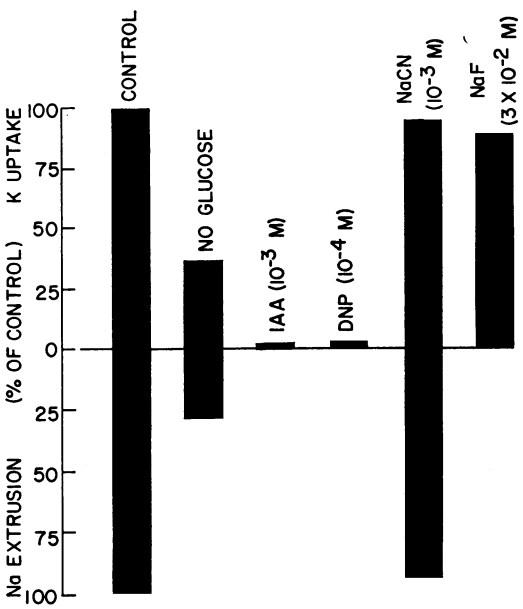


FIG. 4. *Effect of glucose deprivation and metabolic inhibitors on net Na and K movement (24).*

coli, these fluxes appear to be completely uncoupled.

Concept of Membrane Carriers

It is not unexpected that processes associated with ionic movements show characteristics which resemble enzyme reactions. Most transport processes show saturation kinetics, ion discrimination, competitive inhibition, and counterflow, all of which indicate that the transported ions during transport are somehow associated with a membrane component. These components, for the most part, have only conceptual existence at the present time. However, one component probably involved in the active transport of Na^+ ions has been purified from the membrane fractions of higher cells (17, 26, 27). It shows "adenosine triphosphatase" activity, and the unusual features, distinguishing this activity from other "adenosine triphosphatase" activity, of requiring both Na^+ and K^+ ions for maximal activity as well as a remarkable sensitivity to the glycoside, ouabain, a specific inhibitor of the process of active extrusion of sodium ions (10). A Na-K dependent "adenosine triphosphatase" has been sought, but not found, in microorganisms (8). Ion uptake believed to represent carrier-mediated transport has been studied in a number of microorganisms. However, the most extensive studies have been carried out with bakers' yeast and *E. coli*. In bakers' yeast, as in the majority of cells studied, K^+ and Na^+ ions are distributed against their electrochemical gradients, and K^+ ion accumulation (as well as Na^+ ion extrusion) is an energy-dependent process (20). In a study of K^+ ion accumulation by bakers' yeast, Armstrong and Rothstein (1) presented evidence that the alkali metal ions as well as H^+ ions are transported by a common carrier system. From kinetic studies and the pattern of competitive inhibition among the alkali metal ions and H^+, they were able to assign a "relative affinity" of these ions for the membrane carrier (Table 3).

The inhibition of K^+ transport by H^+ ion is seen in Fig. 5. From a comparison of the slope and intercepts of the curves for *p*H 2.0 (10^{-2} M $[H^+]$) and *p*H 3.5 ($10^{-3.5}$ M $[H^+]$), it can be seen that, over this range of H^+ ion concentration, H^+ ion is a competitive inhibitor. However, a further analysis of the effect of H^+ ion concentration on K^+ transport shows that, between 10^{-5} and 10^{-4} M, the H^+ ion acts as a noncompetitive inhibitor (compare the curves for *p*H 3.5, 4.5, and 6.7 and 8 in Fig. 5). From these data, the authors inferred that there are present on the carrier molecule two sites with which H^+ ions may combine.

Only one of these sites is the "transport" site, namely, the site with which the transported cation must combine for transport to be effected. H^+ ions may compete with the alkali metal ions for this site. However, there is an additional site on the carrier with which H^+ ions (as well as other cations) may combine. When this second site is occupied by an H^+ ion (which it is at an H^+ ion concentration greater than 10^{-4} M or at a *p*H below 4.0), then the maximal rate of carrier transport is reduced. These authors refer to this second site as a "modifier" site. The "affinities" of other cations for this site are also given in Table 3. Note that the "affinities" of each ion for the transport

TABLE 3. *Affinity constants of the transport site and the modifier site for cations*

Ion	Transport		Modifier	
	Concn	Relative	Concn	Relative
	mM		mM	
H^+	0.3	0.4	0.05	0.02
Li^+	20	30	20	7
Na^+	15	20	20	7
K^+	0.7	1	3	1
Rb^+	1.3	2	—	—
Cs^+	12	17	4	1
Mg^{++}	300	400	8	3
Ca^{++}	>1,000	>1,000	3	1

and modifier site may differ markedly. This is especially so for Mg^{++} and Ca^{++}, which are not transported to any significant extent by the alkali metal carrier (6). The most important consequence of the existence of two distinct sites with different affinities is the fact that, depending upon the concentrations of metal ions or the H^+ ion concentrations, there will be a different order of cation discrimination. The manner in which the order of cation preference may be changed is illustrated in Fig. 6 and 7, which show how the parameters of ion discrimination, namely, the K_m and the V_{max}, are altered by changes in the hydrogen ion concentration.

These data show how the concept of membrane carriers can provide an understanding of the mechanism of selective ion accumulation in biological systems. The mechanism of coupling of metabolic energy to carrier transport to accomplish ion movements against electrochemical differences has been reviewed recently (27, 30); the subject is beyond the scope of this review.

Ionic Accumulation and Exclusion by Intracellular Binding Sites

Up to this point it has been assumed that, because of the correlations between ion distribution and the electrical potential, the activity coefficients of the ions inside and outside the cell are identical, or nearly so. If, however, the ion activities are

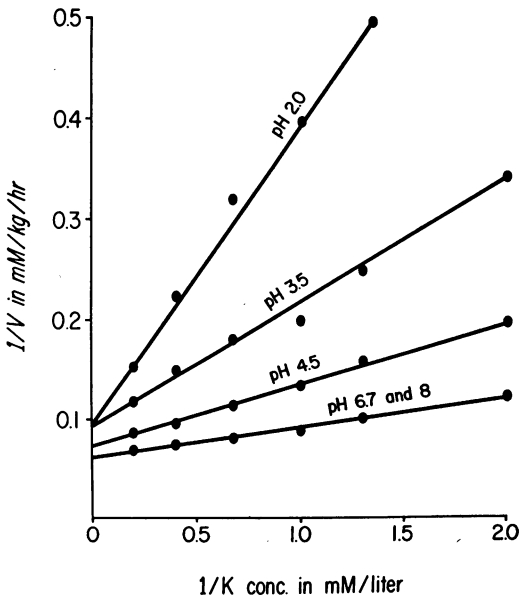


FIG. 5. *Double reciprocal plots of the effect of the H^+ ion concentration on K^+ uptake by bakers' yeast (1).*

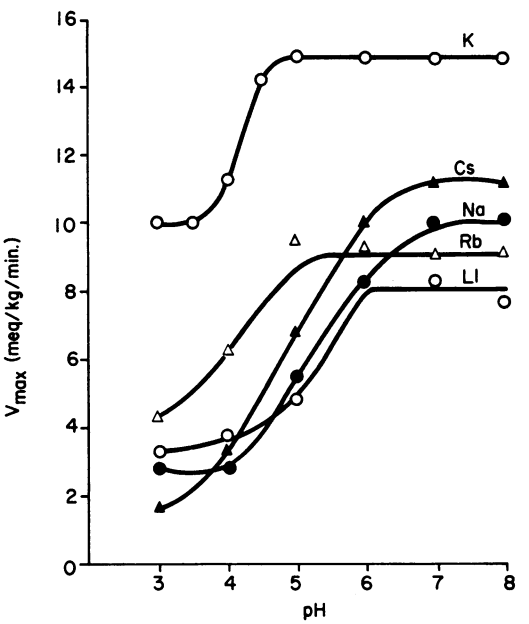


FIG. 6. *Effect of pH on the V_{max} of alkali metal transport in bakers' yeast (1).*

significantly different on the two sides of the cell membrane, then the conclusions based on this assumption are invalid. The correspondence between ion distributions and electrical potentials predicted from the Nernst equation would then have to be reinterpreted. Others have emphasized this in their efforts to interpret ion accumulation as the result of intracellular binding of ions to metabolically controlled sites rather than as a result of the activity of membrane carriers. These interpretations are sometimes referred to as "sorption" theories, the concept of which is indicated by Cowie and Roberts (7) as follows, based on their early work on *E. coli* and *Torula utilis*: "The protoplasm may be likened to a sponge, the cell membrane to a surrounding hair net unable to exclude the entrance or emergence of small molecules. Into this system water may diffuse freely and carry with it many of the dissolved substances which it contains. Thus the nutrients of the environment diffuse into the reactive sites of the protoplasm and become nondiffusible."

Entropy of ion associations in a fixed-charge system. For understanding the role of ion binding in accumulation of elements in cells, there is need for consideration of the thermodynamic factors that determine anion-cation associations (13, 14, 16).

The extent of anion-cation association for the reaction:

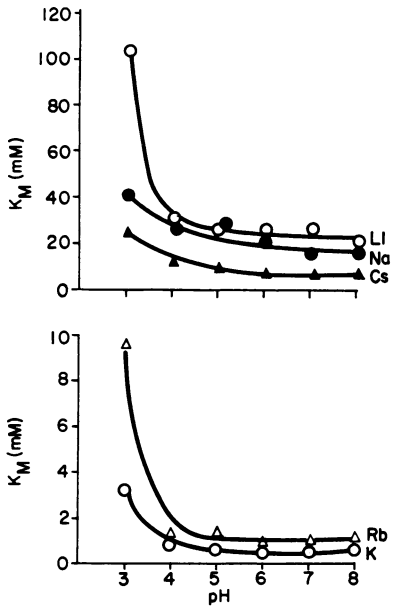


FIG. 7. *Effect of pH on the K_m of alkali metal transport in bakers' yeast (1).*

$$n\ \text{Anion}^{m-} + m\ \text{Cation}^{n+} \rightarrow \text{Anion}_n\text{Cation}_m\ \text{complex} \quad (14)$$

depends upon the association constant, K:

$$K = [\text{Anion}_n\text{Cation}_m]/[\text{Anion}^{m-}]^n[\text{Cation}^{n+}]^m \quad (15)$$

From classical thermodynamics we know that the equilibrium constant depends upon the change in free energy, ΔF, associated with reaction 14 according to:

$$\Delta F = -2.3RT \log K \quad (16)$$

From the second law of thermodynamics we also know that the change in free energy associated with a reaction such as shown above (occurring in solution and at constant temperature) is expressed by:

$$\Delta F = \Delta E - T\Delta S \quad (17)$$

The second law states that only reactions which lead to a loss of free energy occur spontaneously. How far the reaction goes depends upon the difference in internal energy, ΔE, and the difference in the entropy, ΔS, between the product and the reactants. If the product of the reaction, the association complex (*nAmC*), has less energy than the reactants, the dissociated ions ($A^{m-} + C^{n+}$), then ΔE is negative and the system goes toward the associated state (i.e., $E_{assoc} - E_{diss} = -\Delta E$). Besides loss of internal energy ($-\Delta E$), a reaction is also favored if it goes from a less random (or statistically less probable) state to a more random (or statistically more probable) state; ΔS is an index of the difference between the randomness (or statistical probability) of the final state and that of the initial state. [Usually F refers to the Gibbs function, which would be given by: $\Delta F = \Delta H - T\Delta S$. The right side of equation 17 refers to the Helmholtz free energy usually designated by A, which would give: $\Delta A = \Delta E - T\Delta S$. However, the difference between ΔH and ΔE is negligible for ion associations occurring in solution at constant pressure and temperature, and equation 17 is, therefore, valid.]

An increase in entropy ($S_{final} - S_{initial}$) = $+\Delta S$) also favors a spontaneous change. Since ΔS is multiplied by $-T$ in equation 17, a positive change in entropy will make ΔF negative. Since the two conditions which favor a spontaneous chemical reaction, namely, a decrease in internal energy ($-\Delta E$) and an increase in entropy ($-T\Delta S$), both result in a negative ΔF; the *sine qua non* of a spontaneous reaction is that there results a decrease in the free energy. (This is a restatement of the second law of thermodynamics.)

With the meaning of ΔF defined, we may proceed with an inquiry into the conditions which affect anion-cation associations in biological systems.

We may rewrite equation 16 in a more useful form:

$$\log K = -\Delta F/2.3RT \quad (18)$$

This equation indicates that, if the association reaction is spontaneous, that is, ΔF is negative, then the right-hand term of equation 17 is positive, since $-(-\Delta F) = +$. Thus, K must be greater than 1, which is what would be expected for a spontaneous reaction. However, how much greater than 1.0 would depend upon how large $-\Delta F$ would be. Equation 15 can be changed to equation 19 by replacing ΔF by the definition of ΔF given by equation 17.

$$\log K = -\Delta E/2.3RT + \Delta S/2.3R \quad (19)$$

As has been stated before, the entropy term of equation 19 is a measure of the probability of occurrence of the final state compared with the probability of occurrence of the initial state. This relationship is expressed by:

$$\Delta S/2.3R = \log P_{assoc}/P_{diss} \quad (20)$$

where P_{assoc} is the number of equally probable configurations that can be assumed by the association complex (*nAmC*), and P_{diss} is the number of equally probable configurations that can be assumed by the ions when they are dissociated. Substituting this definition into equation 19 gives:

$$\log K = -\Delta E/2.3RT + \log P_{assoc}/P_{diss} \quad (21)$$

This equation may be converted to the more useful, exponential form:

$$K = P_{assoc}/P_{diss}10^{-\Delta E/2.3RT} \quad (22)$$

The association constant is, therefore, determined by the ratio P_{assoc}/P_{diss} (or the partition function), which represents the entropy factors of the reaction, and the exponential expression, which represents the energy factors of the reaction.

It will be useful to analyze the influence of the entropy and energy factors separately. Since the probability function relates to the relative probability of occurrence of the associated state and the dissociated state, association is usually small in dilute solutions. This is due in part to the fact that the fraction P_{assoc}/P_{diss} is very small, because by becoming associated both ions lose translational freedom. One might state that the complex represents a less random state. This decrease in randomness (or entropy) will, therefore, not favor association. However, Ling (13–15) emphasized that the entropy factor is different in ionic associations when one of the ions is fixed in a three-dimensional lattice. In such a case, one must consider the volume which is available to the dissociated ions as compared with the volume available to the complex. As long as the volume for the dissociated state exceeds the volume occupied by the associated state, the ratio P_{assoc}/P_{diss} will be very small and the association constant (see equation 15) will also be small. If, however, the fixed charges are very close together but uniformly distributed so that their association sites do not overlap, as shown in Fig. 8, then the relative volume occupied by the associated ions may be equal to, or even larger than, the volume available to the dissociated counter cation plus fixed anion. In the latter case, there may actually be an entropy gain upon association. Ling indicated that the extent of association may also be favored by decrease in the dielectric constant of water between ions separated by only one or two water molecules. The dielectric constant of bulk water is very high, namely, 80, which decreases attraction between charged groups to $\frac{1}{80}$th that which would exist in air at the same distance. However the first water molecule, and to some extent the second water molecule, around a cation has a reduced dielectric constant, possibly as low as 1. However, the importance of the dielectric saturation effect on ion association in biological systems has been challenged by Conway (4, 5).

Selectivity of ion accumulation. A combination of the entropy factors which Ling suggested for the fixed-charge system, plus the possible effect of dielectric saturation on ion associations in a tightly ordered lattice, can account for the accumulation of ions by biological structures. However, the selectivity of this accumulation requires further analysis of the energy factors associated with anion-cation combinations. Ling (13, 14) and Eisenman (9) calculated the association energies for the combination between oxyanions of various strength and alkali metal cations and H^+ ions. These data are represented in Fig. 9, in which the ordinate represents the association energy, ΔE.

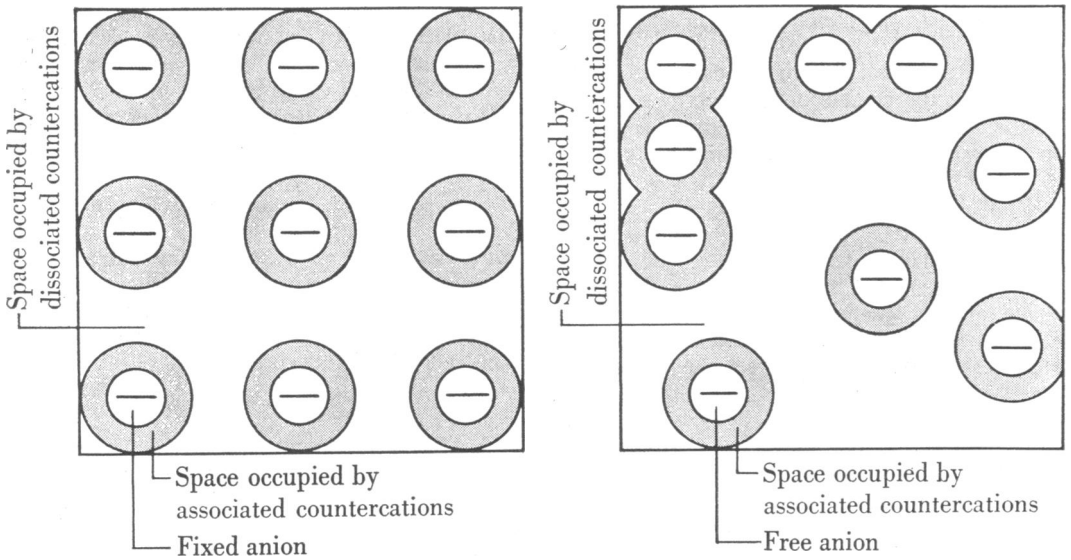


FIG. 8. *Effect of configurational entropy on ion association in a fixed-charge system and in free solution (14).*

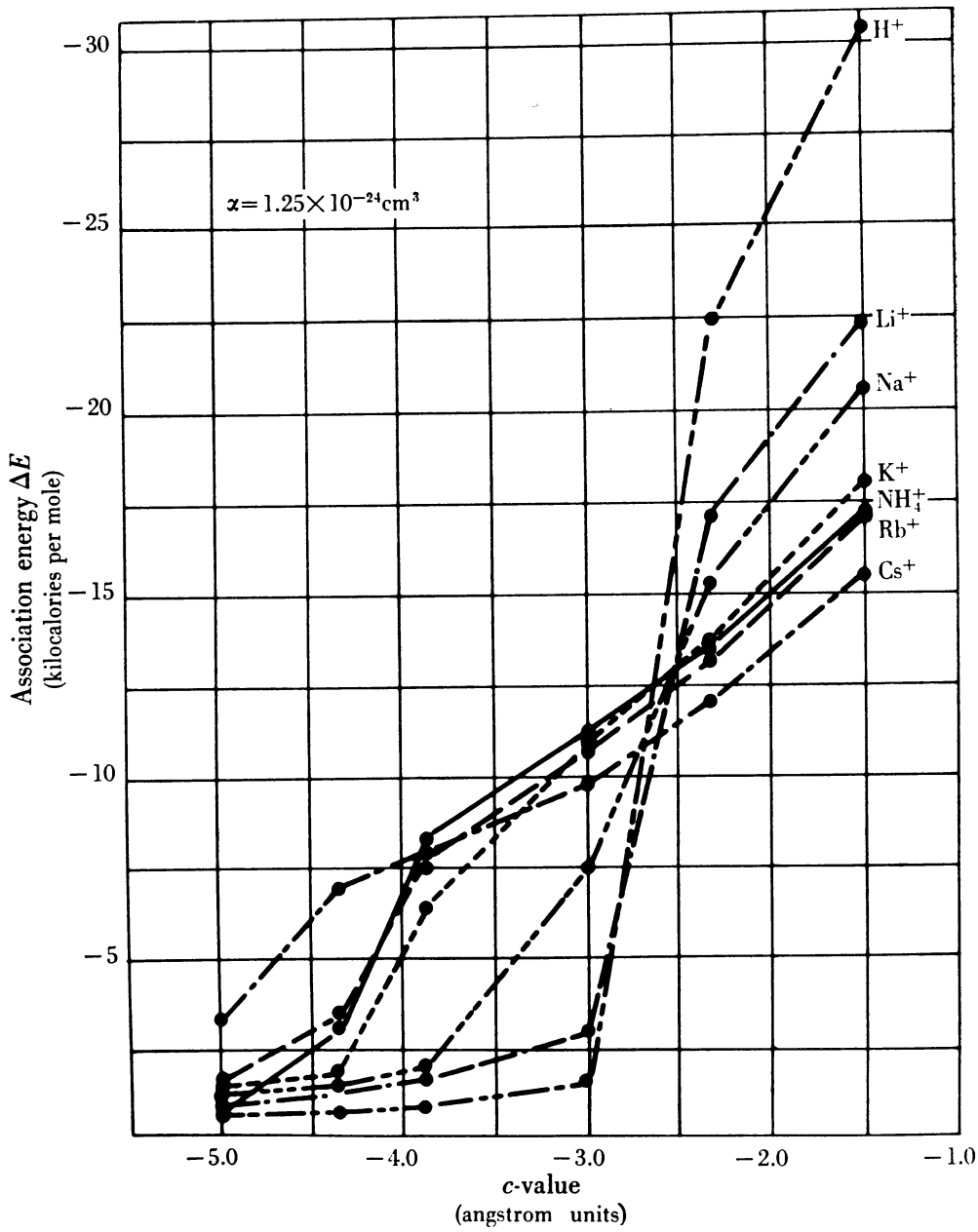


FIG. 9. *Relation between the calculated association energy, ΔE, of various cations and the strength of the anion group (14).*

The more negative the association energy, the more stable the association will be. This is shown in equation 22, in which the equilibrium constant for association is shown to be proportional to $\exp_{10} - \Delta E/2.3RT$. When ΔE is negative, the exponent is positive and K becomes greater than 1.0. When ΔE is positive, the exponent is negative and K is less than 1.0. The abscissa represents the oxyanion strength, which increases from left to right. A c value of −3.0 corresponds to an oxyanion of the strength of trichloroacetic acid; a c value of −1.0 corresponds to an oxyanion of the strength of acetic acid. Therefore, for any cation, the strength with which it is held by an

oxyanion and the extent to which association is favored increases from left to right. Thus, ΔE changes from near zero values to relatively high negative values in going from left to right. The selectivity of an oxyanion of any given strength for the cations included in the figure decreases from top to bottom. Thus, for an oxyanion with a c value of -3, the order of selectivity is NH_4^+, K^+, Rb^+, Cs^+, Na^+, Li^+, H^+; for an oxyanion with a c value of -1.0, the order of selectivity is H^+, Li^+, Na^+, K^+, NH_4^+, Rb^+, Cs^+. The inversion of the order of selectivity with change in the oxyanion strength is the most significant result of this analysis. There is a remarkable similarity between the inversions of selectivity shown by Fig. 9 and those described by Armstrong and Rothstein (Fig. 6) for the effect of *p*H on alkali metal uptake by bakers' yeast.

Surface potentials. A central question in the context of this symposium is whether the fixed-charge system can account for the observed "membrane potentials" (and their reversal when this can be observed, such as in muscle and nerve). Ling derived equations to predict the electrical potentials, and he reported that surface potentials which were predicted by the equations were consistent with experimental observation. His general equation:

$$\psi = 2.3RT/F \text{ constant} - \log (K_K [K^+]_o + K_{Na} [Na^+]_o) \quad (23)$$

may take the form of the classical Nernst equation, and predicts that the surface potential, ψ, is proportional to the absolute temperature and to the log of both the K^+ and Na^+ ion concentrations. However, under appropriate conditions—that is, when changes of oxyanionic strength result in alterations of the affinity constant for K binding (K_K) or Na binding (K_{Na})—this equation reduces to a Nernst-type equation for a K^+ or a Na^+ diffusion potential (13–15).

CONCLUSIONS

The controversy between the membrane and sorption theories will undoubtedly be resolved by some "vigorous hybridization." The virtues of Ling's and Eisenman's concepts are self-evident. The concepts indicate what must happen at cell surfaces or in any other region of the cell where the kind of lattice structures they describe occur. However, it is doubtful that this is the exclusive structure throughout the protoplast. In fact, there is evidence against this concept (5). Nonetheless, Ling and Eisenman have provided what will probably prove to be an atomistic basis for carrier selectivity upon which most transmembranal transport depends. In this respect, the discrimination of the alkali metal carrier in bakers' yeast, described by Armstrong and Rothstein, shows remarkable similarity to the alterations of ion selectivity described by Ling and Eisenman. (Reviewing the arguments over whether there are or are not membrane carriers for transport across the cell membrane is reminiscent of the story of the theistic and atheistic goldfish swimming around in the fishbowl discussing whether there is or is not a God. When the discussion reached the inevitable impasse, the theist goldfish threw up his fins and asked: "If there is no God, who changes the water?")

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